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(54) Composition for metastasis prevention.

(57) The invention relates to pharmaceutical compositions intended to decrease the incidence of tumor metastasis in patients who suffer from malignant diseases.

The pharmaceutical compositions of the invention contain as active ingredient heparin or a suitable derivative thereof. Amongst suitable derivatives are N-desulfated and N-acetylated heparin.

The dosage of the administered heparin or heparin derivative is quite critical and will generally be in the range of from 0.05 mg/kg/day to about 0.5 mg/kg/day. A preferred range is between about 0.1 mg/kg/day to about 0.5 mg/kg/day.

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COMPOSITION FOR METASTASIS PREVENTION

FIELD OF THE INVENTION:

The invention relates to medications for use in the therapy of malignant diseases. More specifically, it relates to means adapted to decrease the incidence of tumor metastasis. The pharmaceutical compositions comprise an effective dosage of heparin, which is quite critical, or of an effective derivative thereof.

BACKGROUND OF THE INVENTION

The process of metastasis, the dissemination of tumor cells to sites in the body distant from the original site of the tumor, often involves invasion of blood vessels by the tumor cells. The blood vessel wall includes a dense extracellular matrix (ECM) of connective tissue that must be pentrated by any cell entering or leaving the vessel. The ECM includes a proteoglycan scaffold that constitutes a physical barrier to cell penetration.

It was found by the research group of Vlodavsky (Vlodavsky, J. Fuks, Z. and Schirrmacher, V. In: The Endothelial Cell - A Pluripotent Cell of the Vessel Wall. Eds. Thilo-Korner, D.G.S. and Fresney, R.I., Basel: Karger, pp. 126-157, 1983;. Vlodavsky, I., Fuks, Z., Bar-Ner, M. and Schirrmacher, V. Cancer Res. 43: 2704, 1983) and of Nicolson (Nakajima, M. Irimura, T., DiFerrante, D., Ferrante, N. and Nicholson, G.L. Science 220: 611, 1983) that tumor cells that were highly metastatic expressed an enzyme, heparanase, that attacked the heparan sulfate moiety of the ECM proteoglycans. Tumor cells that were less metastatic expressed less heparanase enzyme. Heparanase activity was also associated with the capacity of non-tumor cells such as T lymphocytes to move through blood vessels.

In view of the above, we have considered the possibility that inhibitors of heparanse enzyme activity might handicap the movements of cells into and out of blood vessels, thereby obstructing the metastasis of tumor cells leading to prolongation of life. Experiments in this direction have confirmed that positive results can be obtained, as set out in the following.

SUMMARY OF THE INVENTION:

According to the invention there are provided pharmaceutical compositions adapted to decrease metastasis dissemination in mammals, including humans. The compositions contain a predetermined quantity of the effective agent, which dosage is quite critical. The active ingredient is heparin or an effective derivative thereof, such as N-desulfated or N-acetylated heparin. The dosage is in the range of about 0.05 mg/kg/day to about 0.5 mg/kg/day of the active ingredient, preferably about 0.1 mg/kg/day to about 0.3 mg/kg/day.

1. Table 1 shows the effect of the administration of heparins on the ability of heparanase to degrade the heparan sulfate in ECM. It can be seen that intact heparin and N-desulfated, or N-acetylated heparin, but not totally desulfated heparin, are active as inhibitors of heparanse activity.

2. Inhibition of Tumor Metastasis by Heparins

Table 2 shows the results of treating mice with heparins on metastasis of 3LL Lewis lung carcinoma cells. C57BL/6 mice were implanted in a hind footpad with 3LL tumor cells and the local tumor was amputated when it reached a size of 8 mm. Two weeks later the number of lung metastases were counted. It can be seen that total desulfated heparin failed to reduce the number of lung metastases. However, treatment with 5 μg of intact heparin or N-desulfated, N-acetylated heparin, reduced by about one half the number of lung metastases. A higher dose of N-desulfated, N-acetylated heparin (50 μg) did not give better results, and actually seemed to allow formation of a greater number of metastases. Thus, a dose of about 5 μg/mouse (0.25 mg/kg) was optimal in preventing metastasis. This indicates that the dosage of heparin is very important.

3. Modified Heparin Treatment prolongs Survival of Mice challenged with EL-4 Tumor Cells

Table 3 shows that treatment of mice with N-desulfated, or with N-acetylated heparin, prolongs life from 16 to 19 days (highly significant by the Wilcoxin Rank Order Test). EL4 injected intraperitoneally is thought to kill mice by metastasizing. Therefore, heparin treatment can prolong life, probably by means of reduction of metastasis (Table 2).

4. Reduction of Metastasis of Melanoma Cells by Administration of Heparin

C57BL/6 mice were inoculated intravenously with $5x10^4$ B16 melanoma cells and 18 days later the mice were killed and their lungs examined for the number of metastases. The results in Table 2 indicate that heparin treatment markedly reduced the number of lung metastases. Therefore, similar to the 3LL and EL4 tumors, the B16 melanoma is susceptible to treatment.

Conclusions

1. Low dose heparin treatment of humans causes a decrease in DTH reactions. This was shown in the animal studies to be due to inhibition of heparanase and T lymphocyte migration to the site of antigen.

2. Treatment of diseases such as rheumatoid arthritis appear to be effective.

TABLE 1.

Heparins inhibit heparanase activity

Inhibitor (1 µg/ml)	Inhibition of heparanase activity
None	No
Heparin	Yes
Heparin: N-desulfated N-acetylated	Yes
Heparin: Total Desulfated	No

Heparanase activity was tested in the presence of heparins at a concentration of 1 µg/ml as described by Vlodavsky, I. et al. In: Extracellular Matrix: Structure and Function 283-308, 1985). N-desulfated, N-acetylated heparin and totally desulfated heparin was prepared as described (Ayotte, L. and Perlin, A.S. Carbohydrate Res. 145: 267, 1986). Inhibition of heparanase activity was detected by failure to obtain ^{35}S-labeled heparan sulfate degradation products.

TABLE 2.

Inhibition of 3LL lung metastases by heparin and modified heparin

Treatment	ug	No. of metastases	Median
Saline	-	TMTC,TMTC,17,15,5	17
Heparin:			
Total desulfated	5	TMTC,20,19,15,14	19
Heparin:			
N-desulfated			
N-acetylated	5	10,9,8,6,4	8
	50	6,9,10,15,17	10
Heparin	5	14,10,9,6,4	9

C57BL/6 female mice, 2 months old, received $3x10^5$ 3LL (Lewis lung carcinoma) cells in a hind foot pad. When the tumor reached a diameter of 8 mm, the foot was amputated painlessly above the knee and 14 days later the mice were sacrificed and the lungs examined for the number of metastases. Groups of mice were treated from the beginning of the experiment by subcutaneous injections of saline (control) or heparin (Leo, Denmark) N-desulfated, N-acetylated or total desulfated heparins prepared as described (Ayotte, L. and Perlin, A.S. Carbohydrate Res. 145: 267, 1986). TMTC = too many to count.

TABLE 3.

Prolongation of survival of mice injected with EL4 tumor cells by treatment with modified heparin.

Treatment	EL4 tumor Day of death	Median
Saline	16,16,16,16,16	16
Heparin: N-desulfated N-acetylated	17,18,18,18,19,19,19,19,20	19

C57BL/6 female mice, 7 months old, were inoculated interperitoneally with 10^4 EL4 tumor cells. One day earlier and daily until death, the mice received subcutaneous injections of 5 µg of heparin: N-desulfated, N-acetylated. The day of death from lung metastases was recorded.

TABLE 4. Reduction of lung metastases of B16 melanoma cells

Experiment 1.		
No of mice	Heparin (u.g daily)	No. of lung metastases
4	0	30±8.5
5	5	14.7±4.9
5	20	16.6±4.8
5	50	18.8±3.5
Experiment 2.		
9	0	4.4±0.4
9	20	1.1±0.1
7	100	0.7±0.1

Similar results were obtained with equivalent doses of N-desulfated and with N-acetylated heparin.

Claims

1. A pharmaceutical composition for decreasing the incidence of metastasis of tumor cells, which composition inhibits heparanase activity, comprising an effective quantity of heparin or of a derivative thereof.

2. A composition according to claim 1, where the compound used is heparin, N-desulfated heparin or N-acetylated heparin.

3. A composition according to claim 1 wherein the daily dosage is of the order of from 0.05 to about 0.5 mg per kg weight per of the patient per day.

4. A pharmaceutical composition according to claim 3, where the dosage is between 0.1 to about 0.3 mg/kg/day.

5. Use of heparin or of a derivative thereof for the preparation of pharmaceutical compositions for decreasing the incidence of metastasis of tumor cells.

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	CHEMICAL ABSTRACTS, vol. 102, no. 25, 24th June 1985, page 437, no. 219099x, Columbus, Ohio, US; M. BAR-NER et al.: "Sequential degradation of heparan sulfate in the sybendothelial extracellular matrix by highly metastatic lymphoma cells" & INT. J. CANCER 1985, 35(4), 483-91 * Abstract * ---	1-5	A 61 K 31/725 A 61 K 31/73
X	CHEMICAL ABSTRACTS, vol. 101, no. 15, 8th October 1984, page 30, no. 122695e, Columbus, Ohio, US; J.R. DRAGO et al.: "The evaluation of heparin in control of metastasis of Nb rat androgen-insensitive prostate carcinoma" & ANTICANCER RES. 1984, 4(3), 171-2 * Abstact * ---	1-5	
X	CHEMICAL ABSTRACTS, vol. 94, no. 25, 22nd June 1981, page 34, no. 202592w, Columbus, Ohio, US; M. MINCHEVA et al.: "Effect of dextran and carboxymethyldextran on healthy and tumor-bearing animals" & ONKOLOGIYA (SOFIA) 1980, 17(4), 189-92 * Abstract * ---	1-5	TECHNICAL FIELDS SEARCHED (Int. Cl.4) A 61 K 31/00
X	CHEMICAL ABSTRACTS, vol. 90, no. 1, 1st January 1979, page 60, no. 560r, Columbus, Ohio, US; C. TODORUTIU et al.: "The effects of heparin on the evolution of experimental metastases" & REV. ROUM. MORPHOL., EMBRYOL. PHYSIOL., MORPHOL. EMBRYOL. 1978, 24(2), 157-60 * Abstract * --- -/-	1-5	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	07-03-1988	SCARPONI U.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
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T : theory or principle underlying the invention
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X	J. CELL BIOCHEM. SUPPL., vol. 10, part A, 1986, page 53, ref. no. A141; T. IRIMURA et al.: "Chemical and biological characterization of semi-synthetic inhibitors against metastatic melanoma cell-derived heparanase" * Abstract A141 * ---	1-5	
X	CHEMICAL ABSTRACTS, vol. 105, no. 11, 15th September 1986, page 27, no. 90871y, Columbus, Ohio, US; I.D. GOLDBERG et al.: "The in vivo effects of heparin fractions on the development of lung metastasis in murine fibrosarcoma" & ANN. N.Y. ACAD. SCI. 1986, 463(COLLOQ. BIOL. SCI., 2nd, 1984), 289-91 * Abstract * ---	1-5	TECHNICAL FIELDS SEARCHED (Int. Cl.4)
X	CHEMICAL ABSTRACTS, vol. 105, no. 17, 27th October 1986, page 32, no. 14508r, Columbus, Ohio, US; D.M. SYLVESTER et al.: "Role of heparin in tumor metastasis" & PROC. WEST. PHARMACOL. SOC. 1986, 29, 121-3 * Abstract * --- -/-	1-5	

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P,X	BIOCHEMISTRY, vol. 25, no. 18, 9th September 1986, pages 5322-5328, American Chemical Society; T. IRIMURA et al.: "Chemically modified heparins as inhibitors of heparan sulfate specific endo-beta-glucuronidase (heparanase) of metastatic melanoma cells" * Whole article; in particular page 5322: title and abstract * ---	1-5	
P,X	DIALOG INFORMATION SERVICES, Data Base 155: MEDLINE 66-88/March, Access no. 06135487; N. SAVION et al.: "Murine macrophage heparanase: inhibition and comparison with metastatic tumor cells" & J. CELL. PHYSIOL. JAN. 1987, 130 (1) p77-84 * Abstract * ---	1-5	
P,X	CHEMICAL ABSTRACTS, vol. 106, no. 17, 27th April 1987, page 26, no. 131349a, Columbus, Ohio, US; D.R. COOMBE et al.: "Analysis of the inhibition of tumor metastasis by sulfated polysaccharides" & INT. J. CANCER 1987, 39(1), 82-8 * Abstract * -----	1-5	TECHNICAL FIELDS SEARCHED (Int. Cl.4)

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